3685-Pos Board B546
Optical Label-Free Biodetection Based on the Diffraction of DNA Molecular Gratings for In Vitro Diagnostic
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Development of biodetection techniques has become the matter of intense research in the field of bioassays. Modern DNA microarrays now target in vitro diagnostics and allow simultaneous identification of several hundred biomarkers. Thus, targeting complex diseases due to the deregulation of several genes can be reached. However some technological bottlenecks slow down the blooming of this technology. Our research focuses on microarray improvement for in vitro diagnostic by implementing nanotechnology processes in order to reduce the cost of multiplexed analysis. For immobilizing the probe molecules at the surface of the biochip, we selected a modified microcontact printing technique which enables us to generate patterns of probe DNA fragments of arbitrary shape and dimensions while preserving the capability of multiplexing in one printing step. Due to its submicrometric resolution, this biopatterning method has been used to generate periodic arrays of DNA probe molecules (1micron pitch). We demonstrated that these molecular gratings efficiently diffracted light from a laser beam. We exploited the changes in the diffracted intensity of these gratings to perform a label-free optical biodetection. In this contribution, using a modified scanner system capable of collecting at high speed the diffracted intensity, we could perform detection of specific DNA fragments (from 25pb to 320pb). Based on this principle, we show that we are able to detect a change of signature due to DNA hybridization. This biodetection method should allow detection of specific gene targets from an analytical solution and will solve the technological bottleneck of target labeling required for fluorescence read out. Test validation of this technology focuses on a dedicated DNA microarray used for screening validated genetic signatures for breast cancer diagnostic as a new medical tool helping the orientation of therapies.

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Single Protein Molecule Detection with a Transfer-APTamer (Piston) Sliding in a Protein Pore
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Aptamers are specific oligonucleotide molecules that bind to various biologically important target molecules. Nanopores are powerful tools for the identification and detection of single molecules, such as DNA, RNA and proteins. α-hemolysin nanopore is utilized to sense the single protein molecule with a transfer-aptamer (piston). The transfer-aptamer (piston) containing polyethylene glycol (PEG) has functions as identified which zone of the beta barrel of pore. Here we report the formation of reversible signature signals using protein engineering, and characterized change the pore conductance depend on special design of transfer-aptamer (piston). The transfer-aptamer was bound with thrombin, which can be trapped to the pore opening and distinguished signature current block compared the thrombin being released from the transfer-aptamer (piston). But, the signal of aptamer only contains DNA which is indistinguishable with or without thrombin. By containing specific tail of aptamer, presence of single protein molecule, we capture the reversible thrombin signature signal using protein pore. The transfer-aptamer (piston) can be used as detector of different ions for nanomachine, nanomaterials, and folding process.

3687-Pos Board B548
Kinetic Dynamcs in an αHl-Based Nanopore DNA Sequencer
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The prospect of DNA sequencing by a nanopore device has received significant attention in the past decade. One suggested approach involves tethering an exonuclease enzyme to an α-hemolysin (αHL) monomer, while the assembled and embedded pore maintains nominal function. In this proposal, processive cleavage of 2'-deoxynucleoside 5'-monophosphates (dNMPs) by the exonuclease enables identification of the cleaved units. This strategy aims to accomplish rapid and robust DNA sequencing by employing time vs. electrical-current recording of individual dNMPs that are sequentially captured at a constriction site located within the nanopore. If achieved, this accomplishment would mark a significant milestone towards the $1000-genome target set by the NIH.
Here we present a Brownian dynamics simulation of the αHL nanopore that mimics the proposed conditions for an exonuclease sequencing device. Analyte motion is described by combining diffusion, electrophoretic drift, and electro-osmotic flow over relevant distances and kinetic time scales, ranging from nanoseconds to microseconds. By implementing the algorithm on a distributed computer network, we achieve sufficient iterations to explore the impact of variable parameters such as dNMP size, pore geometry, applied electric field strength, the pore's ion selectivity, the analyte's electrophoretic mobility, and the analyte's diffusive behavior inside and outside the pore. We also decouple the driving factors that give rise to analyte translation and detection. The data show that thermal motion typically overwhelms unidirectional forces at nanoscale dimensions. The analysis also suggests ways to potentially improve DNA sequencing fidelity.

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Detection of Single Actin Filaments at Fluorescence Interference Contrast Checkpoints
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A number of emerging concepts for on-chip biotechnologies replace microfluidic flow by active, molecular-motor driven transport of filaments. Examples include applications in bio-simulation, diagnostics, and drug screening. Here we employ actomyosin molecular motors, embedded in nanostructures, as a platform for bio-simulation of the time evolution of motile objects in complex networks. A specific need for this type of application is detection of filaments at specific checkpoints in the device with high signal-to-noise ratio, for example to record the number and speed of filaments at a certain location in the device. To serve this need, we make use of fluorescence interference contrast (FLIC) at thin gold lines running perpendicular to nanosized polymer resist channels that guide filament motion. We have demonstrated that it is possible to track single or multiple filaments passing over these gold lines, using either an enhanced or quenched fluorescence signal. We will discuss the fine-tuning of the device design, development of an algorithm for analyzing the optical readout signal from these detectors, and exploration of the error limits of detection. The results will help establish the viability of active, motor-driven on-chip applications which, among other advantages, offer substantial potential for miniaturization due to the absence of a need for pumps. The results also open for automatic read-out of velocity in high-throughput motility assays e.g. for drug discovery or fundamental biophysical investigations. This work is supported by MONAD, an EU-FP7 collaborative effort.

3689-Pos Board B550
Subwavelength Metal Apertures for Label-Free Detection of Single-Molecules
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A central goal in bioanalytics is to determine the concentration of, and interactions between biomolecules. Nanotechnology offers the possibility to perform such analyses in a highly parallel, low cost and miniaturized fashion. Here we report on label-free counting, mobility and volume analysis of single molecules and nanoparticles during their diffusion through a sub-attoliter detection volume, confined by a 100 nm aperture in a thin gold film. A high concentration of small fluorescent molecules renders the aqueous solution in the aperture brightly fluorescent. Non-fluorescent analytes diffusing into the aperture displace the fluorescent molecules in the solution, leading to a decrease of the detected fluorescence signal, while analytes diffusing out of the aperture return the fluorescence level. The resulting fluorescence fluctuations provide direct information on the volume, concentration and mobility of the non-fluorescent analytes through fluctuation analysis in both time and amplitude. In order to fully benefit from the array of holes and its high capacity of parallelization, the device allows wide-field illumination and CCD detection. This arrangement simplifies instrumentation compared to confocal microscopy and opens new possibilities to detect macromolecules and particles of pharmaceutical importance such as viruses.